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BIOLOGICAL BULLETIN

THE AXIAL GRADIENTS IN HYDROZOA. III. EXPERIMENTS ON THE GRADIENT OF TUBULARIA.

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A. INTRODUCTION.

In a recent paper Banus ('18) states that there is no difference between the time of regeneration of oral hydranths on apical and basal pieces of the stem of *Tubularia*. The data presented by Banus apparently support this conclusion. Such data are, however, of no significance unless complete information is given as to the manner in which they are obtained. Perusal of Banus's paper reveals the fact that absolutely no information is imparted concerning the conditions under which the experiments were performed or the manner of handling the material. Those who have worked on the physiology of the lower forms are well aware that experimental results can be readily controlled and modified by conditions. It is, therefore, impossible for the impartial mind to accept the validity of Banus's conclusions, until further information concerning his experiments shall be forthcoming. Grave doubt is cast upon the correctness of Banus's statements by the fact, completely ignored by him, that experiments of this kind had already been performed several times, with results contrary to his. In addition to these omissions, Banus has made a number of exaggerated and misleading statements.

Banus begins by saying that Child "assumes the existence of metabolic gradients in a great number of species of animals and plants and on this assumption he builds a theory of individuality." In view of the great mass of data which has been presented concerning metabolic gradients and the extent to which these

data have been checked by several different methods it seems hardly scientific to dismiss the matter under the word "assumes." Gradients are not assumptions; they are facts. It is legitimate, of course, for any one to question and criticize the interpretation of these facts, and desirable that other possible explanations of them should be suggested; but to ignore such facts by designating them as assumptions is not the way to arrive at scientific truth. The nature of the axial gradients has been so thoroughly and frequently discussed in numerous papers from this laboratory that presentation of the subject here seems to me superfluous.

Banus next remarks that the hydroid *Tubularia* is extensively used to support Child's conceptions. It is scarcely necessary to point out to the zoölogical world the exaggeration conveyed by this statement, as it is well known that other cœlenterates have been used as extensively and other lower forms much more extensively in accumulating the experimental evidence upon which those conceptions rest.

We are next informed that Child "has made no measurements of the rate of metabolism of different regions of the stem of *Tubularia*." We would be pleased to carry out such experiments if Banus would kindly suggest a suitable method. The matter would be relatively simple were it not for the fact that the relative proportions of perisarc and cœnosarc vary in different regions of the stem of *Tubularia*. It is therefore difficult or impossible to determine the amount of living material in portions of the stem and impossible to establish any basis for comparison of the metabolism of different regions. Naked hydroids, such as *Corymorpha*, would be required for experiments of this kind. The metabolic rate of different regions of the first zoöid of *Planaria dorotocephala* has been determined and has been found to accord with the metabolic gradient conception.

Banus then proceeds to discuss the regional differences in rate of regeneration of *Tubularia*. He says that Child "assumes" the existence of such differences. In his summary he states that "the rate of regeneration of the oral hydranth of an apical piece is on the average identical with the rate of regeneration of the oral hydranth of the basal piece"; and further that "there is no evidence of the existence of level or regional

differences in the stem of *Tubularia*." Such statements as these display an unpardonable ignorance of the literature dealing with the regeneration of *Tubularia*. This form has been investigated by a number of well-known zoölogists, and twenty years ago the regional differences which Banus denies were demonstrated to exist.

The first experiments on the regeneration of *Tubularia* were those of Loeb ('91 and '92), performed at Naples. In the first of these publications (on p. 15) he states that he took very long stems, cut off the roots and polyps, and then divided the remaining portion in half. No difference was observed in the rate of regeneration of the oral hydranths on the apical and basal pieces.

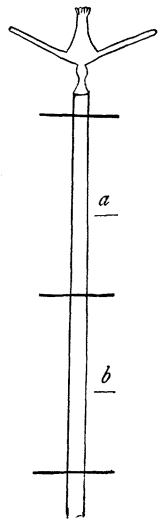


FIG. 1. Diagram of *Tubularia* to illustrate method of cutting apical and basa pieces of the stem of equal length; employed in experiments recorded in Tables III., IV., V., VII., IX., and X. *a*, apical; *b*, basal.

The following year he repeated the experiment and this time he observed that in one experiment (he does not state how many experiments were performed) the oral hydranths emerged about twenty-four hours earlier on the apical than on the basal pieces. Owing to the meager details which are furnished regarding these experiments, it is impossible to determine why the same investigator working on the same material should at one time obtain

one result and on another occasion the contrary result. Loeb has chosen to disregard the one contrary experiment since later ("Organism as a Whole," p. 171), he again asserts, in spite of all the evidence at that time available to the contrary, that apical and basal pieces of the stem of *Tubularia* regenerate hydranths simultaneously. This statement has never been confirmed, except by Banus; every other zoölogist who has worked upon the matter has found the contrary to be true. In 1899, Driesch working also at Naples expressed himself as fully convinced that Loeb was mistaken. He found many evidences of regional differences in *Tubularia*. He observed that the length of the primordium is greater the nearer the piece is to the original distal end of the stem; that in very small pieces, the more apical pieces produce larger hydranths and tend to give rise to distal structures only, while the basal pieces produce smaller hydranths and proximal structures; and that when long pieces of the stem are cut in half, the apical halves give rise to oral hydranths earlier than the basal halves. Driesch, therefore, as he emphatically stated in this paper, disagreed with Loeb on this point. In Table X, p. 131, Driesch gives a record of thirty pieces in which the apical halves regenerated oral hydranths one to twenty-three hours earlier than the basal halves in twenty-five cases and simultaneously with them in but five cases. Table XI, p. 132, presents similar data. These statements of Driesch were verified by Morgan ('01, '05, '06a, '08), and by Morgan and Stevens ('04). Thus Morgan says ('05, p. 496),—"the rate of both oral and aboral development is determined by the level at which the end lies." Again in 1906, p. 497, he states: "It has been shown in *Tubularia* that the time required for the formation of a new hydranth depends on the distance of the cut surface from the old hydranth. The nearer the cut surface to the oral end the quicker the regeneration. The same law also holds for the development of the aboral hydranth from the aboral end of a piece." These statements were reiterated in 1908, p. 157—physiologically polarity is "shown in the more rapid regeneration of the cut surfaces the nearer they are to the distal end."

Child ('07) again performed the experiment in question and

agreed with the results obtained by Driesch, Morgan, and Stevens. These experiments of Child's are the only ones considered by Banus and the false impression is thereby conveyed that no one but Child had ever experimented upon the matter. Banus has criticized these experiments of Child's on the grounds that they are not extensive and that the differences recorded are in some cases so slight that they may be due to experimental error. It

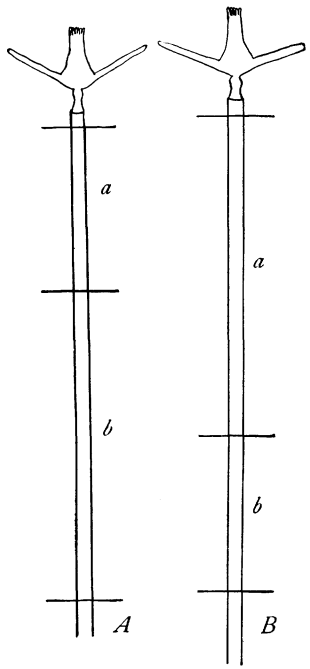


FIG. 2. Diagram to show method of cutting unequal pieces for the experiments given in Table VI. 2A, apical pieces half as long as basal pieces; 2B, apical pieces twice as long as basal pieces.

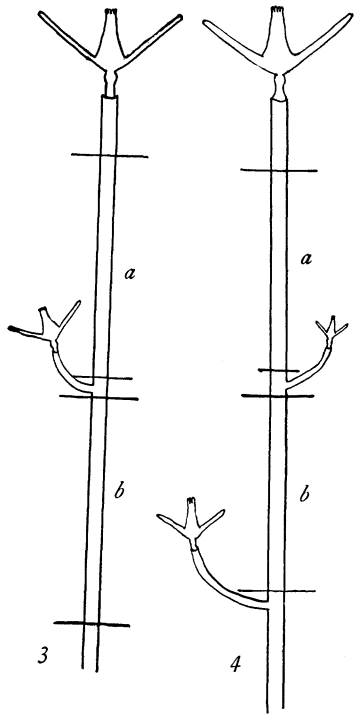
is true that no very extensive series of experiments were carried out because Child felt that the point had already been settled by the work of Driesch and Morgan and that any further experimentation was superfluous. Such observations as he made were therefore incidental to other matters. It is also true, as Banus says, that the differences obtained were not very large, but since they always vary in the same direction, the results cannot be due, as claimed by Banus, to experimental variation. In Table

I., p. 2, of Child's paper we find that of 24 pairs of pieces, the distal oral hydranths emerged first in 14 cases, at the same time as the proximal oral hydranths in 5 cases, and later than the latter in 5 cases. A result which is due to experimental error should vary equally in both directions. It should also be stated that part of the pieces given in this table were regenerating in modified sea-water and not under normal conditions. In regard to Tables II. and III., p. 5, Banus has misrepresented the facts. In these tables the differences between the times of emergence of distal and proximal oral hydranths are less than in the preceding table, although they still vary in the same direction; but it is distinctly stated by Child in the text that the difference is decreased owing to the smaller size of the pieces, and he further shows that with still greater reduction in the length of the pieces, the proximal oral hydranths will emerge first. In short, these experiments were directed towards demonstrating the effect of reduced length on the time of emergence of the oral hydranths, a fact which Banus in quoting them omits to mention.

After Banus's paper appeared the experiment was repeated at Woods Hole in the summer of 1918 by Dr. W. C. Allee. Dr. Allee was entirely unable to agree with Banus's statements, but found, on the contrary, that the oral hydranths arise earlier on apical than on basal pieces of the stem of *Tubularia*. He communicated this result to Professor Child and other members of this laboratory and also showed his experiments to a number of people at Woods Hole. In the summer of 1919, Dr. Allee assigned the experiment to his class in Invertebrate Zoölogy at Woods Hole. Twenty-seven sets of experiments were performed by the students, each set consisting of from two to eight pairs of pieces. Twenty-four hours after cutting, the apical halves (as indicated by the red color of the regenerating ends) were in advance in twenty-five of the sets, the basal halves in advance in one set, and in the other, there was no difference. Of the 112 pairs of pieces cut, 95 of the apical pieces survived, and 71 of the basal pieces. After forty-eight hours, 52 or 55 per cent. of the apical pieces had produced hydranths, while this had occurred in only 15, or 20 per cent. of the basal pieces. It should be stated that the material was not in first-class condition at the

time, and, as I shall show, the difference between the time of regeneration of apical and basal pieces is reduced under such circumstances. Professor Child and I are greatly indebted to Dr. Allee for his interest in the matter, and for his kindness in putting through the experiments.

In view, therefore, of the overwhelming preponderance of the evidence already at hand in support of the existence of the



FIGS. 3 AND 4. Diagrams to show method of cutting pieces for experiments given in Table VIII. Figure 3, method for all experiments in Table VIII., except number 36; figure 4, method used for experiment 36.

regional differences along the axis of *Tubularia* which are denied by Banus, further experimentation seems superfluous. Under the circumstances, however, a repetition of the experiment has been deemed necessary by various members of this laboratory as an answer to Banus's paper. I therefore undertook to repeat his work and for this purpose made trips to Woods Hole in June, and in December, 1919. The results were identical at both

seasons of the year and were completely at variance with Banus's statements. I was unable to verify any of his results. In numerous experiments conducted for this purpose, the apical halves of *Tubularia* stems regenerated markedly faster than the basal halves of the same stems. I further believe that I discovered the cause of Banus's peculiar results. My results are presented in detail in the present paper.

Not only are the researches just enumerated opposed to Banus's statements but a large number of other facts concerning the regeneration of *Tubularia* clearly point to the existence of axial differences in metabolic rate in this form. Thus all of the facts collected by Driesch, Morgan, and Child concerning the phenomena of "polarity" in *Tubularia* are entirely irreconcilable with the view point of Banus and Loeb. If there is no axial difference along the stem of *Tubularia*, why should the apical end of a piece produce a hydranth and the basal end a stolon, or if a hydranth, only later than the apical end? Why do heteromorphic hydranths arise simultaneously on the two ends of very short pieces while on long pieces the aboral hydranth is delayed? This question has received no adequate answer except that based on the axial gradient conception; in short pieces, there is practically no gradient, and hence each end of the piece begins to produce a hydranth at the same or nearly the same time; while, in long pieces, the apical end by virtue of its higher metabolic rate gets the start in hydranth formation and hence gains control of the stem for a certain distance, thus inhibiting the formation of the aboral hydranth. Why, as shown independently by Driesch, Morgan, and Child, in an axial series of very short pieces, do the apical pieces produce larger distal structures with much reduced or absent proximal structures while the more basal pieces give rise to smaller distal structures and larger proximal parts? Longer pieces from the distal region thus resemble shorter pieces from the proximal region in the structures which they produce, owing to the pronounced tendency of the distal pieces to use up their substance in the formation of distal structures only. Why is the primordium of the oral hydranth larger in apical than in basal pieces and the emerged hydranth likewise larger? These and numerous similar facts

have been repeatedly ascertained by several investigators and are totally inexplicable on the point of view maintained by Loeb and Banus that there are no regional differences in the stem of *Tubularia*.

There is one further statement made by Banus to which we must take exception. This is the assertion on p. 266 and again on p. 273 that regional differences in rate of regeneration constitute the "actual basis" for the axial gradient conception as

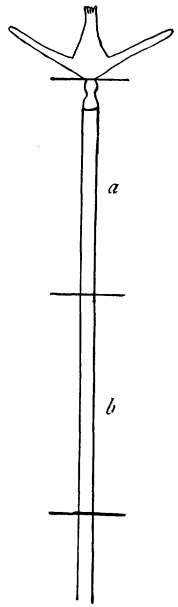


FIG. 5. Method of cutting pieces usually employed by Banus and used for the experiments recorded in Table XI.

applied to *Tubularia*. They are part of the basis, but not the entire basis, as Banus implies. Axial differences in metabolic rate along the stems of *Tubularia* had been demonstrated at the time when Banus published his paper by two other methods—the differences in susceptibility to potassium cyanide and other substances, and the differences in electric potential. The results yielded by both of these methods are again considered in this paper.

In view of the fact that Banus's results are in total disagreement with a considerable number of researches, a critical examina-

tion of his method of procedure is necessitated. But, as has already been pointed out, this is impossible because no description is given by Banus concerning his material, his methods, or his experimental conditions. We need to know the season of the year, the temperature, the vegetative condition of the material, the length of time material was kept in the laboratory before being used, the presence or absence of lateral buds on the stems employed (since buds mark the limits of the individual), and particularly the level from which the pieces were taken, with reference to the original hydranth. We have repeatedly pointed out that metabolic gradients are not fixed and static things, but markedly dynamic and labile, and particularly in the lower forms, they may result from external conditions and may be readily modified and altered by conditions. The failure of Banus to describe or consider the various factors mentioned, any one of which might alter the experimental result, is evidence that he really does not understand the metabolic gradient conception and has not interested himself in understanding it. This is further shown by certain remarks made in his paper such as for example the naïve statement on p. 268 that the pieces were "long enough to show a marked difference according to Child's opinion," whereas in fact "according to Child's opinion," and as even a hasty perusal of the work put out from this laboratory would show, the axial differences are most clearly marked in most respects in relatively short pieces.

In order, therefore, to obtain any information concerning the experiments of Banus, it has been necessary for me to communicate with him. Mr. Banus replied to the first letter which I wrote to him, but did not reply to two others requesting further details. It is therefore not possible for me to furnish all of the details necessary for a correct evaluation of these experiments but the information I was able to obtain is sufficiently astonishing. Banus states that his experiments were performed in New York City in November and in Woods Hole in December. Since most of the other researches on *Tubularia* were carried out in the summer season, it was at first thought that seasonal differences in the vegetative condition of *Tubularia* might account for Banus's results. I found, however, that *Tubularia* is in prac-

tically the same condition at Woods Hole in June and in December and yields identical results at these two seasons of the year. Banus further states that the "temperature during regeneration was in New York 22° C., and in Woods Hole it was about 15° C." Upon examination of Banus's tables, one finds that the time interval between section and emergence of hydranths is much too great for these temperatures. At a temperature of 22° C., apical pieces of *Tubularia* produce hydranths as early as 36 hours after section and the great majority of such pieces will have completed regeneration in 48–60 hours, yet in no case do any of the pieces recorded in Banus's tables regenerate in less than 53 hours and the majority of them require more than 60 hours. Even at a temperature of 15° C., the times given by Banus are surprisingly long, since I found that at 12° C., the majority of the apical pieces will regenerate within 70–80 hours (see Table IV.). It is therefore evident that in Banus's experiments some factor is acting to delay the time of regeneration of the apical pieces, whose regeneration precedes by an average of 10 to 12 hours, according to my findings, the regeneration of the basal pieces.

The cause of the delayed regeneration in Banus's experiments lies in all probability in his method of cutting the apical pieces. In reply to my inquiry concerning the level of the stem from which he cut the pieces, Banus made the following statement: "the most distal cut was usually made as near as possible to the hydranth without including any part of it." Such a method of procedure explains the aberrant results obtained by Banus. It has long been known that the short stalk below the hydranth of *Tubularia* is incapable of regeneration. When the apical pieces are cut in the manner described by Banus, this stalk forms the distal end of such pieces. It dies and disintegrates, thereby markedly delaying the regeneration and time of emergence of oral hydranths on these pieces. I shall present evidence in this paper (see Table XI.) that when the apical pieces are cut in such a way that their distal ends are just below the base of the original hydranth, the time of emergence of the oral hydranth is greatly delayed and falls behind that of the basal pieces. Hence when such a procedure is followed, all

sorts of irregular results are obtained and it is not surprising that under such circumstances, the experimenter would be lead to question the existence of axial differences along the stem of *Tubularia*. When the correct method of cutting the apical pieces is observed, namely, when the hydranth, its stalk, and the first millimeter or two of the stem are discarded, then there is not the slightest question that the apical halves of stems prepared in this manner give rise to oral hydranths very much in advance of the basal halves.

The experiments presented in this paper were performed at Woods Hole in June and in December, 1919. I am greatly indebted to Professor F. R. Lillie for a research room at the Marine Biological Laboratory on both of these occasions and for a grant from the departmental funds covering the expenses of the December trip. I am also indebted to Professor C. M. Child for advice and suggestions throughout the course of the work.

B. Susceptibility Gradients in Tubularia.

The death gradients in lethal concentrations of various substances have already been described by Child ('19*b*) for a number of hydroids including *Tubularia*. I have repeated and confirmed these observations for a number of forms. In *Tubularia*, the disintegration (1/100 to 1/400 mol.KNC) begins at the tips of the proximal tentacles and proceeds down these tentacles to their bases; soon after the proximal tentacles have begun to disintegrate, the process is initiated in the distal tentacles and proceeds to their bases. At about the same time as the tips of distal tentacles begin to die, the mouth region disintegrates and the disintegration extends along the body of the hydranth to its base. In many cases, it was observed that the outer surface of the proximal tentacles (*i.e.*, the surface that contains the most nematocysts) preceded in disintegration the inner surface. In the stalk of the hydranth there is a specialized region bearing a ring of nematocysts; in many cases it was noted that this region disintegrated early and without any definite relation to the progress of disintegration in other parts of the hydranths, as was also noted by Child. Such specialized regions, owing to functional activity, are commonly highly susceptible to toxic agents,

a fact that has been noticed by us on many different forms. It is to be understood that the disintegration of the ectoderm precedes that of the entoderm by a considerable time interval except in the case of the rim of the mouth where both germ layers seem to disintegrate almost simultaneously forming a great bulging mass of disintegrated particles.

The remarks in the foregoing paragraph refer to fully developed hydranths. In young hydranths, it was commonly observed that the distal tentacles disintegrated first and the disintegration then extended basipetally along the body of the hydranth, the proximal tentacles disintegrating later than the body of the hydranth. In medusa-buds, the disintegration proceeds from the free to the attached end. Young hydranths are more susceptible than fully developed ones only after they have reached a certain stage.

The hydranths are much more susceptible than the stems. The disintegration of the stems is obscured by the presence of the perisarc. Nevertheless the progress of disintegration was observed in many cases in the stems but was never followed for more than a short distance. The disintegration proceeds from the base of the hydranth down the stem. It might be said in criticism that the killing agent can gain access to the stem only from the open top of the perisarc and that the death gradient in the stem may therefore be simply a consequence of the diffusion path followed by the agent. To answer this objection short apical pieces of stems bearing hydranths were cut off and their disintegration followed in cyanide. In such cases the open cut surface at the proximal end of the stem affords a readier point of entrance for the reagent than does the top of the perisarc; nevertheless except for a small area of disintegration around the cut surface, the disintegration proceeds in the same manner as before, from the distal end of the stem proximally.

Direct observation of the course of disintegration in the stems is not, however, entirely satisfying, as the process is admittedly obscured by the presence of the perisarc and could be observed for only the most distal region of the stems. Another method was therefore employed, namely, the differential survival of distal and proximal pieces of the stem in nearly lethal concentrations of toxic substances. The substance employed for this purpose

was ether. It was found that distal pieces are more susceptible to ether than proximal pieces, that more of them die when both kinds of pieces are exposed for a certain length of time to a given concentration, and that of those that survive, the regeneration is more delayed in the apical than in the distal halves. An experiment of this kind is recorded in Table I. In this experiment the

TABLE I.

DIFFERENTIAL SUSCEPTIBILITY OF APICAL AND BASAL PIECES OF EQUAL LENGTH (10–12 MM.) TO 2 PER CENT. ETHER.

Exposed to ether for twenty hours. Table records condition of the pieces seven days after cutting. Temp. 12 ± 2° C.

Condition.	Apical.	Basal.
No. of hydranths emerged	5	14
No. of pieces with primordia of hydranth[1]	5	7
No. of pieces living but without primordia[1]	2	15
No. of pieces dead[1]	38	14

hydranths and first millimeter or two of the stems were discarded, the stems were then cut into two equal pieces, each 10–12 mm. long, and all of the distal pieces placed in one finger bowl and the proximal in another. A solution of 2 per cent. ether in sea-water was then poured on both sets of pieces as soon as possible after they were prepared and they were left in this for twenty hours. They were then thoroughly washed and left in normal sea-water. The temperature throughout was 12° C. ± 2. The condition of the fifty pairs of pieces seven days after cutting is shown in the table. The greater susceptibility of the apical pieces is perfectly evident. More of them have died than in the case of the basal pieces and of those that survived, the basal pieces are much in advance in the process of regeneration.

C. GRADIENTS IN REDUCTION OF POTASSIUM PERMANGANATE.

Child (19*a*) has called attention to a new method of demonstrating the metabolic gradients. This method consists in exposing the organisms to appropriate concentrations of a readily reducible substance like potassium permanganate. This substance is reduced by protoplasm, a brown precipitate of manga-

[1] Pieces were examined with the compound microscope. Pieces showing smooth rounded ends and circulation were counted as living, while stems containing only masses of granules were regarded as dead.

nese dioxide being formed. It was found by Child that this capacity of organisms to reduce permanganate exhibits the same kind of a gradation in relation to the body axes as does their time of death in toxic solutions. The apical ends reduce the most permanganate and take on a very deep brown or almost black color and the depth of this color decreases basipetally.

I have observed the staining of *Tubularia* by potassium permanganate. The picture thus presented is identical with the course of disintegration already described. The tips of the proximal tentacles stain first, the tips of the distal tentacles next; the stain progresses rapidly down the tentacles (on the outer surfaces of the proximal tentacles before their inner surfaces) to their bases. Meantime the stain appears on the distal end of the body of the hydranth and progresses basipetally along its ectoderm. After the staining is completed, it is found that the tips of both sets of tentacles and the mouth region of the hydranth are very deeply stained and the stain shades off to the bases of tentacles and hydranth. This was best observed by turning the hydranth with a needle so that it faced upward.

The staining of the stem was naturally difficult to observe. The short stalk below the hydranth became stained soon after the hydranth but the staining of the stem proper was very slow. As far as could be observed the stain proceeded from the distal end of the stem proximally. The observations were, however, unsatisfactory.

The younger hydranths stain much more rapidly than the larger ones, but exhibit less distinct graded differences between different regions of the hydranth.

D. Electrical Gradients in Tubularia.

Differences in electrical potential along the axes of organisms form still another manifestation of the metabolic gradients and are at present being investigated by members of this laboratory for a number of the lower forms. These electrical gradients correspond in all respects to the death and staining gradients. The regions of highest susceptibility and reducing power are electronegative (galvanometrically) to regions of lower susceptibility and reducing power. This matter has been discussed

elsewhere (Hyman, '18) and further papers upon the subject will appear shortly. Briefly it is believed that chemical differences are as a rule responsible for these permanent differences in potential and that such chemical differences arise in the final analysis through differences in metabolic rate at different levels.

The existence of such a difference in electrical potential along the axis of *Tubularia* was discovered by Mathews ('03). Mathews found that the hydranth is negative to any region of the stem and that distal levels of the stem are negative to proximal levels. He correctly attributed these potential differences to differences in metabolic activity.

I have repeated these experiments on *Tubularia* and verified all of Mathews' statements. A galvanometer constructed on the principle of the D'Arsonval galvanometer and put out by the Leeds and Northrup Company was used. Although not as sensitive as some other types of galvanometers in use, the instrument was found adequate for the purpose. Non-polarizable electrodes were employed, made in the usual way of the glass tubes from medicine droppers, packed at the small end with kaolin paste made with sea-water, and filled at the other end with saturated zinc sulphate solution. Zinc rods, amalgamated with mercury, dipped into the zinc sulphate solution, and were connected by copper wires with the binding posts of the galvanometer. Small rolls of hard filter paper were thrust into the ends of the electrodes and the stems to be tested placed on these. These filter paper rolls were kept soaked with sea-water. Although such electrodes are made as nearly alike as possible, there is almost always some difference of potential between them. Such difference increased with use so that it was necessary to renew parts of the electrodes or to make wholly new ones at frequent intervals. The existence of a potential difference between the two electrodes of course makes it impossible to obtain an absolute value of the amount of current originating from the organism; but absolute values were not desired in the present experiments. It was my purpose merely to discover which regions of the organism were electronegative as compared with other regions.

In performing the experiments isolated stems of *Tubularia* in

perfect condition only were employed. They were placed across the filter paper ends of the electrodes and the reading of the galvanometer recorded. The stem was then reversed on the electrodes and the reading again taken. The difference in the two readings, particularly the difference in the direction of swing of the indicator on the scale gives the desired information about the electrical condition of the stems. Each reading was repeated once, sometimes twice. When dead organisms are tested in this way, the galvanometer gives the same reading regardless of the position of the material on the electrodes.

The galvanometric readings obtained on *Tubularia* are recorded in Table II. In connection with this table it is necessary to explain that the scale of the galvanometer used is printed in red on the right side of the zero point and in black on the left side. When the right electrode, which is connected with the right hand binding post of the galvanometer, is negative, the indicator swings to the right of the zero point and hence reads on the red half of the scale; when the left electrode is negative, the indicator reads on the left hand or black side of the scale. In some cases, both readings may be on the same side of the zero point but one farther to the right or left than the other. Left and right refer, of course, to the hands of the observer as he sits facing the instrument.

In the table, each number refers to one individual and all of the data given under that number were obtained on one individual. In Table II., *a*, are recorded the readings of the galvanometer when the *hydranth* is compared with nearby portions of the stem or with distant portions or with regions where branches are present. Table II., *b*, gives the readings when distal portions of the *stem* are compared with more proximal regions or with far proximal regions or with proximal regions bearing branches. In each table, the first column gives the number of the individual, the second describes the material, the third gives the reading in one position between regions not very far separated, the fourth column the readings for the same regions in the reversed position on the electrodes, the fifth and sixth are the same as the third and fourth except that they compare regions more widely separated or distal regions with levels bearing branches.

TABLE II., *a*.

ELECTRICAL GRADIENTS OF *Tubularia;* HYDRANTH COMPARED WITH NEARBY PORTIONS OF THE STEM AND WITH FAR PROXIMAL PORTIONS OR REGIONS BEARING BRANCHES.

Each number refers to one individual. Scale of the galvanometer reads with red figures to the right of the zero point, with black figures to the left of zero, hence *r* means red, and *b* means black, and numbers accompanying *r* and *b* are divisions on the scale. The right electrode is negative when the reading is on red or farther to the right than before; and the left electrode is negative when the reading is on black or farther to the left than before. Hydr., hydranth, br., region of branches, prox., proximal, dist., distal.

No.	Material.	Readings with Reference to Position of Material on Electrodes as Designated in These Columns.			
		Hydr. Right, Stem Left.	Hydr. Left, Stem Right.	Hydr. Right, Far Prox. or Br. Zone Left.	Hydr. Left, Far Prox. or Br. Zone Right.
1.	Hydr. and stem	5 *r*	7 *b*		
		3 *r*	7 *b*		
2.	Small hydr. and stem	2 *b*	4 *b*		
		2 *b*	2 *b*		
3.	Hydr., stem, and br.	0	15 *b*	0	8 *b*
		2 *r*	10 *b*	3 *r*	5 *b*
5.	Ditto	2 *r*	5 *b*	0	4 *b*
		0	5 *b*	0	4 *b*
6.	Large hydr., stem and br.	4 *r*	8 *b*	2 *r*	6 *b*
		5 *r*	8 *b*	2 *r*	6 *b*
8.	Small hydr. and stem	0	3 *b*	0	2 *b*
		0	5 *b*	0	2 *b*
10.	Large hydr., stem and br.	2 *r*	6 *b*	0	5 *b*
		2 *r*	9 *b*	0	5 *b*
11.	Large hydr. and stem	0	5 *b*		
		2 *b*	6 *b*		
12.	Medium hydr. and stem	3 *r*	6 *b*		
		0	5 *b*		
13.	Ditto	0	4 *b*	2 *b*	0
		0	4 *b*	2 *b*	0
14.	Ditto	0	6 *b*		
		1 *r*	4 *b*		
15.	Ditto, and br.			0	5 *b*
				1 *r*	5 *b*
16.	Ditto			1 *b*	3 *b*
				0	3 *b*
17.	Small hydr. and stem	2 *b*	4 *b*		
		0	2 *b*		
18.	Medium hydr. stem and br.	5 *r*	2 *b*	2 *r*	5 *b*
		0	6 *b*	2 *r*	2 *b*
19.	Small hydr. and stem	0	3 *b*		
		1 *r*	2 *b*		
21.	Very large hydr. and stem	13 *r*	18 *b*		
23.	Medium hydr. and stem	3 *r*	4 *b*		
		5 *r*	4 *b*		
35.	Ditto	0	14 *b*		
		6 *r*	15 *b*		

TABLE II., *a*, *Continued.*

No.	Material.	Readings with Reference to Position of Material on Electrodes as Designated in these Columns.			
		Hydr. Right, Stem Left.	Hydr. Left, Stem Right.	Hydr. Right. Far Prox. or Br. Zone Left.	Hydr. Left, Far Prox. or Br. Zone Right.
37.	Ditto, and br.....	7 *r*	19 *b*	2 *r*	6 *b*
		4 *r*	10 *b*		
38.	Medium hydr. and stem.....	13 *r*	1 *r*		
		5 *r*	0		
39.	Ditto, and br.....	7 *r*	0	2 *b*	2 *b*
				0	1 *b*
40.	Medium hydr. and stem.....	8 *r*	18 *b*	9 *r*	6 *b*

The tables give all of the readings which were made. There have been no omissions or selection of data. Forty-two individuals in all were tested. The first forty of these came from the same lot of *Tubularia*, collected on December 6, and tested on December 7 and 8. The last two individuals came from another lot of material collected on December 8 and tested on the same day. Material was kept at a temperature of 10° C. A number of different readings were commonly made on each stem, various levels of the stem being tested in order to obtain a picture of the potential differences along the whole organism.

The following conclusions may be drawn from the data presented in Tables II., *a*, and *b*:

1. The hydranth is always electronegative to nearby regions of the stem. This is shown without exception in the twenty-three cases given in Table II., *a*. The galvanometer invariably reads to the right when the hydranth is on the right electrode, and to the left when the position is reversed.

2. The difference between hydranth and distal regions of the stem is greater in the case of larger hydranths and much less in the case of small hydranths. Thus in nos. 2, 8, 17, and 19, where small hydranths were used, the potential difference between hydranths and stem is 2 to 5 points of the scale; while when medium or large hydranths are used, the differences are much greater. These conditions are probably associated with age.

3. Hydranths are usually more negative to distal portions of the stem than to more proximal regions or regions where lateral

TABLE II., *b*.

ELECTRICAL GRADIENTS OF *Tubularia*.

Distal regions of stem compared with proximal or with far proximal or region bearing branches. Otherwise as in table II., *a*.

No.	Material.	Readings with Reference to Position of Material on Electrodes as Designated in These Columns.			
		Dist. Stem Right, Prox. Left.	Dist. Stem Left, Prox. Right.	Dist. Stem Right, Far Prox. or Br. Left.	Dist. Stem Left, Far Prox. or Br. Right.
1.	Hydr., stem and br.			2 *b*	4 *b*
				2 *b*	4 *b*
4.	Stem without hydr., hydr. regenerating	1 *b*	5 *b*	0	0
		1 *b*	5 *b*	1 *b*	1 *b*
		1 *b*	5 *b*		
7.	Stem without hydr., like 4.	0	3 *b*		
		0	3 *b*		
9.	Stem without hydr., like 4.	1 *r*	5 *b*	2 *b*	0
		0	3.5 *b*	2 *b*	0
13.	Medium hydr. and stem.	4 *b*	2 *r*		
		5 *b*	0		
20.	Ditto	3 *b*	5 *b*		
		2 *b*	10 *b*		
		2 *b*	8 *b*		
22.	Ditto	4 *b*	5 *b*		
		3 *b*	4 *b*		
24.	Ditto	3 *b*	5 *b*		
		2 *b*	5 *b*		
25.	Ditto and br.			2 *b*	1 *b*
				2 *b*	1 *b*
26.	Medium hydr. and stem.	1 *b*	3 *b*		
		1 *b*	3 *b*		
27.	Ditto	2 *r*	4 *b*		
28.	Ditto	No potential difference			
29.	Ditto	2 *r*	1 *b*		
		2 *b*	4 *b*		
30.	Ditto	1 *b*	2 *b*		
		0	3 *b*		
		0	2 *b*		
31.	Ditto	0	2 *b*		
		0	3 *b*		
32.	Ditto	1 *r*	2 *b*		
		1 *r*	4 *b*		
33.	Ditto, and br.			1 *b*	1 *b*
				1 *b*	1 *b*
34.	Small hydr. and stem.	No potential difference			
36.	Medium hydr. stem and br.,			0	2 *r*
				0	2 *r*
41.	Hydr., very long stem.	12 *r*	0		
		12 *r*	5 *b*		
42.	Like 41.	7 *r*	9 *b*		
		8 *r*	9 *b*		

branches are present. This is shown in numbers 3, 5, 6, 8, 10, 15, 16, 18, 37, and 40, Table II., *a*. Thus for example, in no. 3, the difference between the hydranth and the distal stem is 15 divisions of the scale in the first trial, 12 in the second, while the difference between the same hydranth and a more proximal region of the stem where a lateral branch was present was 8 divisions in both trials. In one case, no. 13, the region of branching was negative to the hydranth; in another case, no. 39, there was practically no potential difference between the hydranth and the branching region. We therefore see that proximal regions of the stem are more negative than distal, especially when they bear branches. This is due to the fact that the hydranth dominates only a certain length of stem, and beyond that length physiological isolation has occurred with the formation of a new individual, expressed by the development of lateral branches. Such new individuals like the original one are electrically negative apically.

4. Distal regions of the stem are nearly always electronegative to nearby proximal regions. This was the case in 14 of the 17 cases tested in Table II., *b*. In two cases, nos. 28 and 34, there was no potential difference between two such regions of the stem; in one case, no. 13, the gradient was reversed, the distal region being positive to the proximal region. Such cases as these three account for the fact that occasionally, distal and proximal pieces of the stem regenerate simultaneously, or that the proximal piece may precede.

5. The potential difference between distal and proximal regions of the stem is always very much less than that between hydranth and distal regions of the stem.

6. The potential difference is usually slight or absent or may be reversed between distal regions of the stem and far proximal regions, or regions bearing branches. Of six cases tested, two showed no potential difference (nos. 4 and 33, Table II., *b*); in one case, the distal region was negative (no. 1); and in the other three cases, the far proximal region or branching region was negative to the distal region (nos. 9, 25, 36). This verifies what was said in paragraph 3. These far proximal regions are really beginnings of new individuals and hence are more electronegative than the regions immediately distal to them.

I therefore find, as Mathews did, that within the limits of a single *Tubularia* individual, any distal region is electronegative (galvanometrically) to any proximal region. Since electronegativity is usually associated with a higher rate of oxidative metabolism in organisms, these experimental data constitute strong evidence that there is a metabolic gradient along the axis of *Tubularia*, that the apical end of this axis has the highest rate of activity, and that this rate diminishes proximally.

E. Differences in Rate of Regeneration of Distal and Proximal Pieces of Equal Length.

A large number of experiments were performed with reference to this point with the result that the apical pieces were found to regenerate markedly faster than the basal pieces in practically all cases. A few cases were observed in which the proximal piece regenerated first.

1. *Method of Procedure.*—The method of cutting the pieces was invariably as follows unless specifically stated otherwise. Stems free from branches and filled with cœnosarc throughout their length were removed from the colony and placed on a glass plate. The hydranth and the first millimeter or two of the stem were then removed by a cut and discarded and the basal end injured by removal from the colony also cut off and discarded. The piece of stem was then cut into two equal halves, a distal or apical half and a proximal or basal half. In most cases, unless otherwise stated, such halves were 8–12 mm. long. Figure 1 illustrates the method of cutting the pieces.

After cutting the pieces were handled in two different ways. In the majority of the experiments all of the apical halves were placed in one finger bowl and all of the basal halves in another finger bowl. Such experiments are designated throughout this paper as *mass* experiments. The number of oral hydranths emerged in each finger bowl at a given time was then recorded. In other experiments, which are designated as *individual* experiments, each half was placed in a separate stender dish and the time of emergence of the oral hydranth on each half stem recorded as accurately as possible. In all cases the record was taken only when the hydranth had emerged completely from the perisarc.

Observations were made and the hydranths emerged recorded every two to four hours during the daytime. No observations were made during the night and hence there are in all of the experiments gaps of from six to ten hours for each night period. The first morning observation after such a gap is indicated in all of the tables by an asterisk.

There has been no selection of experiments for presentation in this paper. Practically all of the experiments performed are presented.

2. *Mass Experiments in June.*—These experiments were performed between June 16 and July 9, 1919. The material was in excellent condition up to July 1, when the pieces for the last experiment were cut. There was a great abundance of material, growing rapidly and containing hydranths of all sizes. Material was always cut on the same day as collected since, as is well known, *Tubularia* will not keep in good condition in the laboratory in the summer. The hydranths fall off within twenty-four fours, new ones being subsequently regenerated; further the cœnosarc either dies away in the basal regions or else retreats to other parts of the colonies. The regenerating pieces were kept in the laboratory during the earlier experiments; the temperature was naturally variable and as recorded in the daytime ranged from 15° to 24° C., with probably lower temperatures at night. The later experiments were placed in the refrigerator at a constant temperature of 13° C.

The mass experiments performed in June are recorded in Table III. As already stated, all of the apical halves were placed in one finger bowl and all of the basal halves in another; the two finger bowls were kept under the same conditions. At frequent intervals the regenerating pieces were examined and the number of oral hydranths emerged recorded. The record was taken only when the hydranth had completely emerged from the top of the cœnosarc and had spread its tentacles.

Details of these experiments not given in the table are as follows. Experiment 1 was performed on slender stems; experiments 2 on stout stems; the other experiments were with medium sized stems although there was some variation in the diameter of the stems. Experiments 1, 2, 5, and 10 regenerated in the

TABLE III.

MASS EXPERIMENTS PERFORMED IN JUNE SHOWING RATE OF REGENERATION OF APICAL AND BASAL HALVES OF STEMS OF *Tubularia*.

Pieces 8–10 mm. long. Hrs. means numbers of hours elapsed since cutting; *a*, apical half; *b*, basal half; columns under *a* and *b* give number of hydranths emerged at time indicated. Asterisk indicates first morning observation.

1.			2.			5.			6.			10.			11.			15.			16.			21.		
Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*b.*	*b.*	Hrs.	*a.*	*b.*
*37.5	13	0	*38.5	3	0	*36.5	2	0	47	1	0	38	2	0	60	1	0	63	1	0	63	1	0	*65	9	0
39	15	3	40	6	0	38.5	2	0	50	3	0	40	3	0	64	2	0	66	5	0	67	6	0	68	13	0
40.5	17	3	43	7	2	41.5	4	0	52	5	0	42	7	1	66	5	1	72	15	0	70	8	0	70.5	18	0
42		4	44.5	9	3	44	9	0	*60	12	4	44	9	1	68	8	3	*82	24	8	73	13	1	73.5	20	2
43.5		5	46	10	3	47	11	0	63	15	6	46	14	2	72	19	5	86	25	10	*83	19	10	77.5	24	5
45		8	48	13	5	50	15	2	66	18	6	48	16	6	74	25	7	90		16	86	22	17	79.5	24	7
47		12	50	15	7	52	15	3	69	19	10	50	18	11	*81.5	33	11	93		17	89	22	19	*89	28	15
51		15	52	18	8	*60	20	15	72	21	12	52	22	12	84	35	13	96		20	93	25	23	93	31	17
*61		17	*62	21	18	63	22	18	75	22	12	*58	29	15	86	36	14	*105		21	97		25	95	31	19
			70		18	66	24	19	*83	24	17	60.5	30	15	88	38	16	111		22				98	33	19
			74		20	69	25	23	85	25	18	62.5	35	17	90	38	16	119		24				101	34	21
			76		21	75		23	88		20	64.5	36	19	92	40	19	*130		25				*114	41	26
						*83		23	90		21	66.5	37	25	94	42	20							116	42	27
						90		24	95		22	68.5	38	27	102	46	23							118	43	28
						107		25	97		23	72.5	41	29	107	47	25							120	43	31
												74.5	41	29	110		31							127	43	32
												*82	43	30	112		33							*137	43	35
												84.5	45	32	117		34							143	44	36
												86.5	48	32	*126		35							*162		38
												88.5	50	32	140		37							165		39
												90.5		33	151		38							*188		41
												95.5		34										198		43
												97.5		34												
												*106		36												
												108.5		37												
												117.5		39												

laboratory at variable and generally moderately warm temperatures, from 15° to 24° C. (day records). In experiment 6, the pieces were placed in the refrigerator (temp. 13° C.) for the first twelve hours and in experiment 11 for the first twenty hours after section, and were then removed to laboratory temperature. In experiments 15, 16, and 21 the pieces were kept in the refrigerator (temp. 13° C.) for the entire period of regeneration as the weather had become unfavorably warm by this time. There was some mortality, particularly among the basal pieces, owing probably to the warm weather. In experiment 6, two basal pieces were living but had not regenerated when the experiment was concluded. In experiment 10, where fifty pairs of pieces were cut, seven basal pieces died and four had failed to regenerate when the experiment was discontinued. In experiment 11, three apical halves and twelve basal halves had died or failed to regenerate when the experiment was discontinued. One basal piece died in experiment 21.

The length of the pieces in all of the experiments recorded in Table III. was 8–12 mm. It was not possible to find stems free from branches long enough to give longer pieces in the summer material, owing to the fact that the colonies are growing rapidly and branching extensively at this season. The number of pieces cut in each experiment depended on the number of healthy stems of sufficient length available in the day's collection. Although material was very abundant and large quantities of it were brought in whenever desired, most of the colonies consisted of stems so short as to be useless for the experiments.

3. *Mass Experiments in December.*—According to the statements of Mr. Gray, head of the supply department at Woods Hole, *Tubularia* is most abundant and in excellent condition in the early summer reaching a climax in June. After that, as the weather becomes warm, the colonies die away, the protoplasm withdrawing into the perisarc and apparently passing into a dormant state. In the fall, as the water becomes colder, the colonies begin to grow again, reaching their height in November and December, and then with still colder weather, once more passing into the quiescent state, emerging in the spring. In November, 1919, no *Tubularia* could be found at Woods Hole,

in spite of diligent search by the collectors. It was, however, obtainable early in December in fair abundance, and experiments were performed upon it from December 6 to 16. The colonies at this time were in excellent condition, branching freely and growing rapidly. The general appearance of the material was much the same as in June except that the hydranths attained a larger size than in June and a few lots of material consisted of very long stems, much longer than any observed in June. The majority of the December material, however, was branching so freely that most of the stems were relatively short and in some cases it was necessary to cut pieces less than 8 mm. long. The temperature of the running water in the laboratory in December was 8° C. and it was therefore possible to keep the material for two or three days in excellent condition. Two collections of material were used in the December experiments; one collected on December 6 was cut for experiments on December 6, 7, and 8; the other, collected on December 8, was used on December 8 to 11. All of the pieces were kept on the water tables in slowly running water at a temperature of approximately 12° C., varying, however, for slight periods from 10° to 14°.

The results of the mass experiments performed in December are given in Table IV. In experiment 26, the pieces were about 5 mm. long; in experiment 27, 5–8 mm. long; and in experiments 35, 44, and 47, 10–12 mm. long. The pieces were cut as in Fig. 1, except in the case of experiment 44, in which the basal pieces were cut at the proximal end of long stems so that some 10–15 mm. of stem was removed between the levels of the apical and basal pieces in this experiment. There was no mortality among the pieces. The temperature throughout was 12° C. ± 2.

4. *Conclusions from Mass Experiments.*—The data given in Tables III. and IV. permit us to draw the following conclusions concerning the rate of regeneration of oral hydranths on apical and basal pieces of the stem of *Tubularia* of equal length:

(*a*) Hydranths invariably emerge first on the apical pieces and a considerable number of such pieces will have regenerated before any of the basal pieces have produced a hydranth.

(*b*) At any given time there are in nearly all cases a greater

number of apical pieces with oral hydranths than basal pieces. The difference is always more marked in the early part of the regeneration period; later the basal pieces may catch up with the more tardy of the apical pieces with the result that the number of regenerated basal pieces may in a few cases equal the

TABLE IV.

MASS EXPERIMENTS PERFORMED IN DECEMBER SHOWING RATE OF REGENERATION OF APICAL AND BASAL HALVES OF STEMS OF *Tubularia*.

Hrs. means number of hours elapsed since cutting; *a*, apical half; *b*, basal half; figures under *a* and *b* give number of hydranths emerged at time indicated; asterisk indicates first morning observation.

26.			27.			35.			44.			47.		
Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*
*61	18	7	45	1	0	*60	16	0	52	2	0	61	1	0
63	19	9	51	2	0	62	19	0	58	4	0	65	2	0
65	21	11	53	8	2	64	22	4	61	8	0	67	4	0
67	21	14	55	15	3	66	23	9	*69	16	1	69	5	0
69	22	15	57	25	8	68	29	18	71	18	8	73	12	3
71		17	59	27	19	70	31	25	73	19	10	75	14	3
73		18	*68	40	40	72	35	29	75	22	16	*85	26	14
76		18	70	42	42	74	37	29	77	25	19	87	29	18
*84		20	72	48	44	77	41	30	79	26	20	89	32	20
90		21	74	49	45	*84	44	36	81	27	23	91	33	26
*112		22	76	51	46	87	45	42	83	28	25	95	33	30
			80	53	50	89	46	45	*93	30	27	97	34	31
			83	54	51	91	47	47	105		28	99	35	33
			*92	55	55	93	49	47	*117		29	101	36	33
			94	57	56	95	49	49	119		30	*111	37	36
			96	57	57	97	51	49				119		37
			98	58	57	99		51						
			100	60	57									
			104		58									
			*117		60									

number of regenerated apical pieces (exps. 27 and 35, Table IV.); but in no case are the basal pieces in advance.

(*c*) In all cases the apical pieces complete their regeneration first.

(*d*) When other factors are equal the rate of regeneration is a function of temperature.

(*e*) When other factors are equal, the rate of regeneration is a function of the diameter of the stem. More slender stems regenerate more rapidly than stouter stems. Thus in experiment 1, Table III., the pieces were cut from slender stems bearing small hydranths; those in experiment 2, same table, from stouter

stems bearing larger hydranths, cut at the same time and from the same lot of material. It is perfectly apparent that the more slender pieces regenerate more rapidly, and this was also evidenced throughout all of my experiments. It is probable that this relation of the rate of regeneration to the diameter of the stem is connected with the age of the stem, but since one does not certainly know that slender stems are younger than stouter ones, the matter must be left open at present. Morgan ('06 *b*) found that young stems regenerate more rapidly than old ones and that when the hydranths are removed from the top and lateral branches of a stem, the lateral branches regenerate first. At any rate, these facts dispose of the suggestion which has been made that apical pieces regenerate more rapidly than basal pieces because they are of larger diameter and hence contain more protoplasm. As a matter of fact it is the pieces of smaller diameter which regenerate the more rapidly. Further in slender stems there is no difference in diameter along the stem, and yet the apical halves of such stems regenerate hydranths earlier than the basal halves.

5. *Individual Experiments.*—These experiments were identical with the mass experiments except that each piece was placed in a separate dish and the number of hours required for it to produce an oral hydranth recorded as accurately as possible. The records of the four experiments of this kind which were performed are given in Table V. Experiments 9 and 17 were performed in June at room temperatures; experiments 29 and 45 in December at a temperature of 12° C. ± 2. Pieces were 8–12 mm. long except in experiment 29, where they were 5–8 mm. long. There was some mortality in the June experiments but none in December.

6. *Conclusions from Individual Experiments.*—The results of these experiments lead to the same conclusions as previously stated from mass experiments. Of 122 pairs of pieces in which both pieces regenerated, the apical halves regenerated hydranths first in 111 cases, or 91 per cent.; the basal halves first in 10 cases, or 8 per cent.; and the time of emergence of the hydranth was practically the same in both pieces in one case. Cases where the basal piece preceded in regeneration are indicated by

TABLE V.

RECORDS OF THE TIME OF EMERGENCE OF INDIVIDUAL APICAL AND BASAL HALVES OF THE SAME STEM.

Dagger calls attention to cases where the basal half emerged first; other abbreviations and symbols as before.

No.	Hours Since Cutting. Exp. 9.		No.	Hours Since Cutting. Exp. 17.		No.	Hours Since Cutting. Exp. 29.		No.	Hours Since Cutting. Exp. 45.	
	a.	*b.*		*a.*	*b.*		*a.*	*b.*		*a.*	*b.*
1	43	dead	1	40	*64	1	45	71	1	73	*91
2	*34	*58	2	77	86	2	103	*114	2	*67	77
3	42	75	3	*39	*64	3	65	79	3	60	75
4	44	50	4	*39	53	4	*88	79†	4	73	77
5	41	dead	5	43	77	5	57	73	5	60	73
6	42	dead	6	*39	45	6	56	69	6	*91	73†
7	38	48	7	*39	76	7	54	71	7	79	*67†
8	42	61	8	45	67	8	75	*88	8	59	71
9	38	44	9	68	67†	9	*64	66	9	56	*67
10	40	50	10	*64	74	10	*64	72	10	59	71
11	38	50	11	*64	dead	11	56	74	11	*115	93†
12	42	48	12	70	dead	12	56	76	12	*67	95
13	46	50	13	71	*87	13	53	*64	13	72	74
14	42	48	14	45	*64	14	*64	*87	14	72	73
15	36	50	15	41	53	15	66	*87	15	*66	*114
16	42	50	16	41	dead	16	*64	68	16	*90	78†
17	36	46	17	dead	116	17	68	90	17	*66	74
18	53	dead	18	75	112	18	92	97	18	72	78
19	42	67	Aver.	53	73	19	66	*87	19	68	76
20	36	46				20	*64	70	20	*66	76
21	42	63				21	76	*87	21	72	76
22	36	46				22	70	96	22	68	78
23	42	46				23	70	96	23	68	80
24	38	40				24	68	*87	24	*66	76
26	42	44				25	*64	76	25	68	76
27	42	42				26	*64	72	26	68	*90
28	40	*58				27	68	*112	27	68	72
29	42	63				28	90	95	28	78	74†
30	50	*58				29	73	104	29	68	78
31	48	63				30	66	76	30	58	70
32	65	67				31	72	*112	31	*66	72
33	61	78				32	68	78	32	*66	76
34	63	62.5†				33	114	99†	33	68	76
35	40	46				34	69	*86	34	68	78
Aver.	42	52				35	*86	91	35	72	76
						36	69	75	36	*66	92
						37	75	*86	Aver.	69	77
						38	75	*86			
						39	67	*86			
						40	*86	101			
						41	66	97			
						42	118	103†			
						Aver.	71	83			

Total number of regenerated pairs..... 122
Number of cases where *a* preceded..... 111 or 91%
Number of cases where *b* preceded..... 10 or 8%
Number of cases where *a* and *b* equal..... 1

a dagger in Table V. The average difference between the number of hours required for the emergence of the hydranths on apical and basal halves was 10 hours in exp. 9; 20 hours in exp. 17; 12 hours in exp. 29; and 8 hours in exp. 45. The individual differences range from half an hour to more than forty hours.

It may be inquired why in a small percentage of cases the basal piece precedes the apical piece in regeneration, and why there is such a great variation in the difference between the time of regeneration of the two pieces. It is highly probable that these variations are related to the degree of physiological isolation existent in the basal pieces before they were cut from the stems. It has already been pointed out that a hydranth controls only a certain length of the stem proximal to it and beyond that limit a new individual arises which eventually expresses its presence by the formation of a lateral bud. Now it is evident that such new individuals must exist physiologically before they give morphological expression of their existence by bud formation. It has already been stated that in these experiments the longest obtainable stems free from buds were used. Such stems are the exception rather than the rule since the majority of the material obtainable, particularly in the summer, will furnish only a small proportion of long stems free from branches. It is therefore obvious that the basal regions of such long stems must be in various stages of the process of physiological isolation and branch formation. The nearer such basal regions are to branch formation the more rapidly will they regenerate when isolated and those that are on the very verge of branch formation may conceivably regenerate as rapidly as or even more rapidly than more apical pieces. This matter is referred to again in connection with experiments on the rate of regeneration of basal pieces cut below branches.

7. *Remarks on the Temperature Coefficient.*—It has generally been accepted that when the rate of a biological process increases two to three times with each ten degrees rise in temperature that such a process is chemical in nature. It may be doubted that this line of reasoning is strictly correct. The use of the temperature coefficient to analyze the nature of a biological process involves the unwarranted assumption that such processes

may be purely chemical; but it is very doubtful that they ever are solely chemical in nature, and, of course, equally doubtful that they are ever the consequence of purely physical changes. In all probability biological processes are neither complexes of purely chemical reactions nor purely the resultants of physical changes but they involve both types of changes occurring simultaneously and mutually interacting. On *a priori* grounds, however, it may be accepted that the chemical processes are of paramount importance in living things, since, while substances having physical properties similar to or identical with those of protoplasm exist which are not alive, in no case do non-living materials carry on the chemical reactions characteristic of protoplasm; further, the "signs of life" are chemical or of chemical origin, and protoplasm in which the chemical reactions have fallen to a low level is to all intents dead. Granting, therefore, that chemical reactions play the most important rôles in life processes and that in many cases physical changes are insignificant, it may be valid to draw conclusions from the value of the temperature coefficient. But it must always be borne in mind that the chemical reactions which occur in living things are subject to processes of regulation in the organism. The relation of chemical changes to temperature is therefore in the organism a variable quantity. Thus Behre ('18) found that the rate of respiratory metabolism of *Planaria* is lowered when the animals are maintained at a high temperature and raised when they are maintained at low temperatures. The temperature coefficient for the rate of respiration of *Planaria* is therefore not a fixed value for a certain range of temperature but depends to some extent upon the temperature at which the animals had been living previous to the experimental test. Since such modifications or regulations are known for emulsoid colloids, their behavior at any given time depending upon the conditions to which they had previously been exposed (phenomenon of hysteresis), it is possible that this ability of organisms to modify the rate of processes presumably chiefly chemical with reference to temperature is due to the colloidal substratum in which the chemical reactions take place.

In *Tubularia* similar regulations to temperature are observable.

The rate of regeneration of pieces of the stem of *Tubularia* is, as has long been known, dependent in large part upon the temperature and the temperature coefficient of this process is described as corresponding to that of chemical reasions (Moore, '10). The rate of regeneration is not, however, wholly dependent upon the temperature at which regeneration occurs but is to some degree affected by the temperature at which the particular stems used had been living previous to their utilization. Thus in the experiments recorded in Tables III. and IV., it can be noted that summer material regenerates more slowly at 13° C. than does winter material at 12° C. While other possible explanations of this fact could be suggested it seems reasonable in the light of other results along this line to suppose that this is another case of acclimation to temperature; material living for some time at low temperature has elevated the rate of its chemical processes above that which would result if the material were suddenly lowered to the same temperature from a higher temperature—a procedure usually practised in experiments on the temperature coefficient.

F. Rate of Regeneration of Distal and Proximal Pieces of Unequal Length.

Banus refers to Child's experiments on pieces of unequal length in which Child found that longer pieces will regenerate slightly faster than shorter ones provided the factor of level is eliminated by always making the apical pieces the shorter pieces. Since apical pieces regenerate faster than basal pieces no conclusions could be drawn regarding the effect of length on the time of regeneration unless the apical piece were the shorter. Banus has "repeated" this experiment and claims that the longer piece always regenerates first regardless of level. Here Banus has again misrepresented Child's statements and he has not in reality repeated Child's experiment. Child distinctly states that changes in the length of the piece "produce only very slight or no appreciable differences in time of emergence of oral hydranths provided the length of the piece is above a certain minimum. But with reduction in length below the minimum the appearance of the hydranth is delayed and this retardation increases with

further reduction in length." The apical pieces will therefore regenerate later than the basal pieces only when they are reduced below a certain minimum length. This minimum length is very much less than any used in Banus's experiments. In order to get the result mentioned by Child it is necessary to cut the apical pieces as small as 2 mm. Yet Banus in "repeating" Child's experiments has used no pieces less than 10 mm. in length. In pieces as long as this, length makes very little difference; according to Child's results and my own, the apical pieces will regenerate oral hydranths earlier than the basal pieces, just as when both pieces are of equal size. In his tables 3, 4, and 5, Banus presents data on apical and basal pieces in which the lengths of the pieces were: 10 and 20 mm., 10 and 30 mm., and 10 and 40 mm. Banus found that the longer pieces in all of these cases, regardless of whether they are apical or basal, regenerate oral hydranths slightly in advance of the shorter pieces.

With these results and statements of Banus I am quite unable to agree. There is some truth in the statement that a longer piece will regenerate slightly faster than a shorter piece with apical end at the same level. Yet in the case of apical pieces, the difference between pieces 10 and 20 mm. long is very slight indeed, in fact, practically nil; but it is plainly marked in shorter pieces, say 5 and 10 mm. long. In the case of the basal pieces the difference in time of regeneration between 10 and 20 mm. pieces is somewhat greater but here it must be remembered that the apical end of a basal piece 20 mm. long is in these experiments at a level 10 mm. more distal than that of a basal piece 10 mm. long, and the factor of level again comes into play. In all cases in pieces exceeding 5 mm. in length, the apical pieces will in general regenerate more quickly than the basal pieces, regardless of their relative lengths; and a basal piece twice as long as an apical piece will still regenerate more slowly than the apical piece, notwithstanding the effect of length.

I have repeated Banus's experiment on relatively long pieces of unequal length and the results are given in Table VI. Three pairs of experiments were performed. In one experiment of each pair the apical piece was *half* as long as the basal; in the other

experiment, the apical piece was *twice* as long as the basal. Pieces for the two experiments of each pair were cut from the same lot of stems at the same time and kept under the same conditions. All of the apical pieces were kept in one finger bowl and the basal in another. The method of cutting the pieces for such experiments is given in text-figure 2. All of these experiments were performed in December at a temperature of 12° ± 2. The length of the pieces is stated in the table. No experiments were attempted with pieces in which the ratio of length was 1 : 3 or 1 : 4, as it is difficult if not impossible to obtain a sufficient number of unbranched stems of the requisite length.

The data given in Table VI. show quite clearly that an

TABLE VI.

RECORDS OF MASS EXPERIMENTS WITH APICAL AND BASAL PIECES OF UNEQUAL LENGTH.

Columns under *a* and *b* record number of hydranths emerged at time indicated.

Exp. 32, *a* = 4 — 6 Mm., *b* = 8 — 12 Mm			Exp. 33, *a* = 8 —12 Mm., *b* = 4 — 6 Mm.			Exp. 37, *a* = 8 —10 Mm., *b*=15—20 Mm.			Exp. 38, *a*=15—20 Mm., *b*=8—10 Mm.			Exp. 42, *a*=10—11 Mm., *b*=20—22 Mm.			Exp. 43, *a*=20—22 Mm., *b*=10—11 Mm,		
Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.
56	1	0	58	1	0	*59	18	0	*59	7	0	53	3	0	53	7	0
58	2	0	*67	15	2	61	24	1	61	11	0	55	7	0	55	9	0
*67	11	9	69	16	4	63	35	3	63	17	0	57	11	0	57	10	0
69	13	11	71	18	5	65	41	7	65	22	0	59	18	0	59	12	1
71	15	16	73	21	7	67	47	13	67	24	3	62	20	0	62	17	2
73	17	17	75	22	14	69	49	18	69	28	10	*69	25	25	*69	26	18
75	20	19	77		17	71	49	25	71	28	14	72	26	26	72	28	24
77	21	20	79		19	73	50	27	73	30	18	74	26	27	74	28	26
79	22	22	81		20	76		30	76	32	21	76	26	28	76	29	27
84	23	22	84		21	*83		35	*83	35	34	78	26	29	78	29	29
*91	23	23	*91		22	86		38	86	37	38	82	27	30	82	30	29
94	24	23				88		40	88	38	45	84	29		84		29
104		24				90		41	90	43	46	*96	30		*96		30
						94		43	94	43	47						
						96		44	96	44	49						
						98		46	*108	49	50						
						*108		47	114	50							
						110		48									
						120		49									
						*132		50									

G. ALTERATION OF THE REGIONAL DIFFERENCES IN RATE OF REGENERATION.

apical piece will regenerate faster than a basal piece of twice its length, in pieces at least as long as 5 mm. Although the factor of length is of some consequence, the factor of level is of vastly

greater importance. In experiments 32 and 33, the longer pieces in each case regenerate faster, but the effect of length does not overcome the effect of level, the apical pieces in both experiments regenerating first on the whole. The influence of length is most marked in experiments 32 and 33, where short pieces were employed. It is very little evident in experiments 37 and 38, and 42 and 43, where pieces 10 mm. in length were employed. In fact, in experiment 37, the apical pieces 10 mm. long regenerate slightly faster than the apical pieces 20 mm. long; and in experiments 42 and 43 there is practically no difference. We may therefore say that length is of little consequence in pieces exceeding 10 mm. in length, in agreement with Child's previous statement and in contradiction to the claims of Banus. In all experiments the longer basal pieces regenerate faster than the shorter basal pieces, but it is probable that this effect is one of level rather than of length, because the longer basal pieces have their apical ends at a higher level than the shorter basal pieces.

It may therefore be concluded that in the case of apical and basal pieces of unequal length, the apical pieces will still regenerate more rapidly on the whole regardless of whether they constitute the shorter or the longer pieces, always provided that their minimum length is 5 mm. The level at which the pieces are cut is still the dominant factor in such experiments. In pieces below 10 mm. in size, a longer piece will regenerate slightly faster than a shorter one; but in pieces above 10 mm. length, length is of practically no consequence. Long basal pieces in such experiments regenerate faster than shorter basal ones mainly because their apical ends are at a higher level than the apical ends of the shorter pieces. These results are the contrary of those of Banus whose experiments are invalidated owing to his erroneous method of cutting the apical pieces as discussed at greater length below.

The data already presented incontestably demonstrate that a regional difference in rate of regeneration exists along the axis of *Tubularia*, such that regeneration is the more rapid the nearer the piece lies to the apical end. It may next be inquired whether this regional difference is modifiable under either certain normal conditions or under experimental conditions. To this inquiry

an affirmative answer may be returned. It is possible to modify or eliminate the regional differences in question. The various methods by means of which this was attempted or accomplished are discussed in this section.

1. *The Effect of Cold.*—A number of experiments were performed in which both apical and basal pieces were exposed to a lowered temperature for a number of hours after cutting or during the entire period of regeneration. The pieces were removed from room temperatures (approximately 20° C.) to the temperature of the refrigerator (13° C.) for various periods of time. Although such a proceeding invariably retards the rate of regeneration, the differences between the regeneration of apical and basal pieces were unaltered by such exposure to low temperature. These experiments are therefore included in Table III. (exps. 6, 11, 15, 16, and 21), as showing the typical difference between the rate of regeneration of apical and basal halves. It is highly probable, however, that with very low temperatures, in the neighborhood of zero, the typical difference between pieces of different level would be reduced or eliminated.

2. *Effect of Using Material Kept in the Laboratory.*—Two experiments were performed in June upon material which had been kept in the laboratory aquaria for a week preceding the cutting of the pieces. As is well known under such circumstances, the hydranths of *Tubularia* fall from the stems and new hydranths are subsequently regenerated. Such new hydranths are smaller than and have a lower rate of activity than the original hydranths; it is therefore to be expected that the regional differences along the axis will be reduced in such cases. As already stated only two experiments were performed as the weather had become warm and little material was available. The material for these experiments was collected on June 30 and cut on July 6. The pieces were kept in the refrigerator (13° C.) throughout the regeneration period. The results are given in Table VII. Experiment 21, Table III., furnishes a control for these experiments. It will readily be seen that the differences between the rate of regeneration of apical and basal pieces are plainly reduced as a consequence of the depressing effect of laboratory conditions upon the physiological axis of

Tubularia. This experiment shows that the metabolic gradient of *Tubularia* is not a fixed and permanent gradient in the stem but is readily variable under the conditions of the animal's environment. Experiments such as those of Banus in which no account is taken nor any description given of the conditions of the material or the environment do not therefore merit serious consideration.

TABLE VII.

RECORD OF MASS EXPERIMENTS WITH APICAL AND BASAL PIECES OF EQUAL LENGTH, THE PIECES BEING TAKEN FROM MATERIAL KEPT ONE WEEK IN LABORATORY CONDITIONS BEFORE CUTTING.

Temp. 13° C. Control, exp. 21, Table III., in which the pieces were cut on the same day as the material was collected.

Exp. 24.			Exp. 25.		
Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*
72	1	0	72	4	0
*84	11	5	*84	9	3
87	12	9	87	11	6
94	22	16	89	13	9
96	22	18	94	22	18
*107	33	28	96	23	23
110	33	30	*107	27	26
114	35	33	110	28	28
117	36	33	114	29	28
119	36	33	117	29	30
123	37	35	119	30	
*146		36			
158		37			

3. *The Effect of the Presence of Branches.*—In the consideration of the data on the electrical gradient in *Tubularia* it was pointed out that the control of a *Tubularia* hydranth extends only for a limited distance down the stem and that the stem beyond this limit is more or less differentiated as another individual. This differentiation, at first purely physiological, is later morphologically apparent by the formation of a bud at the level of the apical end of the new individual. The appearance of the bud not only indicates the formation of a new individual but also is an expression of a loss of control of the basal portions of the stem by the original hydranth. It is therefore to be expected that pieces taken above such lateral branches will have a lower metabolic rate than pieces of the same level from unbranched stems; and further that pieces taken below the branch, since

they are near the apical ends of new individuals (the real apical end being the hydranth of the branch) will have a higher metabolic rate than ordinary basal pieces. Owing to the operation of both of these factors it may be expected that the difference between apical and basal pieces will be reduced when they are cut above and below, respectively, the level of a branch. This was found to be the case. Banus in his paper does not state whether or not he used stems free from branches and did not reply to inquiries on this point.

In preparing pieces for this kind of experiment, the following procedure was usually adopted. Stems having one branch at about the middle of the stem were selected, the terminal hydranth; upper millimeter or two, and basal end cut off and discarded as usual. An apical piece was then cut anterior to the branch, and a basal piece of equal length posterior to the branch; the small piece bearing the branch was discarded. As found by Morgan and verified in my experiments, the stumps of lateral branches left on pieces will regenerate hydranths more rapidly than the distal end of such pieces, and these lateral hydranths will then inhibit the formation of the terminal hydranth; hence in experiments of this kind it is necessary to avoid using pieces bearing stumps of branches. The method of cutting the pieces in most of the experiments is illustrated in Fig. 3. In one experiment, stems having two branches were selected, the apical piece cut in front of the first branch, and the basal piece between the two branches as illustrated in Fig. 4.

The results are presented in Table VIII. All experiments of this kind were mass experiments. Experiments 12*a* and 12*b* were performed in June at room temperature, the remaining experiments in December at 12° C. ± 2. The controls for these experiments are indicated at the top of the table; such controls were cut at the same time and from the same lot of material, with the exception that they came from stems without branches, and were kept under the same conditions. The pieces in experiments 28, 30, and 34 were short pieces, 5–10 mm. long; those in experiments 12 and 36, approximately 10 mm. long. The pieces for all experiments except number 36 were prepared according to Fig. 3; those for experiment 36 as in Fig. 4.

The effect on the relative times of regeneration of apical and basal pieces by cutting them above and below a lateral branch is of course slight but nevertheless it is evident in most cases. If the experiments given in Table VIII. are compared with those

TABLE VIII.

RECORDS OF MASS EXPERIMENTS WITH APICAL AND BASAL PIECES OF EQUAL LENGTH, THE APICAL PIECES BEING TAKEN IN FRONT OF THE FIRST BRANCH, THE BASAL PIECES BELOW THE BRANCH.

Columns under *a* and *b* give number of hydranths emerged at time indicated. Controls in Table III. and IV., exp. 10 for exp. 12; exps. 26 and 27 for exp. 28, 30 and 34; exp. 35 for exp. 36.

Exp. 12*a*.			Exp. 12*b*.			Exp. 28.			Exp. 30.			Exp. 34.			Exp. 36.		
Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.	Hrs.	*a*.	*b*.
37	1	0	33	1	0	53	1	0	54	1	0	56	1	0	*59	2	0
43	2	0	37	2	0	55	2	0	*62	4	0	58	3	0	63	4	0
45	3	0	*43	6	2	57	3	0	66	5	2	*67	17	2	65	5	0
49	4	1	47	10	3	60	11	0	68	7	4	69	17	3	67	8	2
51	4	3	49	13	6	*68	13	5	70	9	8	71	17	8	69	11	4
*57	6	4	51	16	9	70	14	6	72	9	9	73	22	9	71	14	10
60	8	4	53	17	13	72	15	7	79	11	10	75	25	14	73	15	11
66	9	5	55	18	15	74	16	9	82	11	13	77	32	20	76	18	12
68	10	6	59	25	19	76	17	11	*90	12	13	79	35	26	*83	28	20
72	11	7	61	28	20	78	18	12	93	13	14	81	38	27	86	35	23
74	11	8	*68	29	22	80	19	13	95	15	15	84	40	30	88	36	27
*81	11	9	71	30	24	83	20	15	99	15	16	*91	55	41	90	43	31
88	12	10	75	31	24	*91	22	18	*116	16		94	57	41	92	47	36
95	13	10	77		25	94		19				96	59	42	94	48	40
105	14	10	85		25	117		20				98		44	96	48	41
108		11	*92		26	119		22				100		47	98	48	43
110		12	97		27							106		51	*108	51	46
			100		28							*116		58	110		48
			107		29							122		59	114		49
															120		51

in Tables III. and IV., it will be found that in general more basal pieces have regenerated in the experimental series when the same number of apical pieces have regenerated in both control and experimental series. This appears chiefly in the early part of the regeneration period. A few such comparisons may be pointed out; in making them it is necessary to select experiments in which the total number of regenerating pieces is similar in experiment and control, since the number of regenerated apical pieces in the early stages of an experiment is greater relative to the basal pieces, the greater the total number of pieces. In experiment 12*b*, Table VIII., 2, 9, and 19 basal pieces have

formed hydranths as compared with 1, 6, and less than 15 basal pieces in the control experiment, number 10, Table III., when 6, 16, and 25 apical pieces have regenerated in both cases. Similarly, in experiment 30, for which experiment 26 is a control, the regeneration of the apical and basal pieces is practically simultaneous, a result which is never obtained when stems free from branches are employed. Comparison of experiment 36, Table VIII., with its control, experiment 35, Table IV., shows the same effect; in the former case 12 basal pieces, in the latter case no basal pieces have regenerated at the time when 18 apical pieces have regenerated in both experiments. In other cases, as in experiment 34, little difference from the control could be observed. The decrease in the time difference between the apical and basal pieces in these experiments is apparently largely due to a delay in the regeneration of the apical pieces. This is to be expected, since as already explained all basal pieces are probably more or less isolated as new individuals, and hence are slightly accelerated in both experimental and control series. The "apical" pieces, on the other hand, in the present experiments, since they are taken in front of the level of a branch, really represent the basal end of the first zoöid of the stem, and hence are delayed in regeneration as compared with pieces similar in position from stems where branches have not yet arisen and where the hydranth still controls most of the length of the stem. In regard to the basal pieces, it should further be pointed out that the really high metabolic point of the new individuals formed at the base of *Tubularia* stems is in the hydranth of the branch, and the basal piece itself below the level of the branch retains only part of the increased metabolic rate after the bud has formed.

Banus has presented one table in which he has compared the rate of regeneration of three equal pieces, each 10 mm. in length, from different levels of the same stem. I have not repeated these experiments as they seem to be lacking in point. The reason why the apical pieces in these experiments of Banus regenerate more slowly than the middle pieces is doubtless, as in the case of the other experiments reported in his paper, the consequence of an erroneous method of cutting the apical pieces. That some

basal pieces may precede the middle pieces is to be expected on the basis of what has already been said. It should be obvious that in stems as long as 30 mm., the length required for these experiments, the basal regions must already be more or less physiologically isolated as new individuals whether branches are present or not. Therefore it may be expected that at least some of these basal pieces will regenerate more rapidly than the middle pieces. It is furthermore to be remarked that the metabolic gradient is steepest near the hydranth and gradually diminishes in slope down the stem; and it has never been claimed by us that any marked axial difference exists along the basal parts of the stems of hydroids. Indeed, we believe that in many cases the gradient has disappeared in these basal regions, as shown by the tendency for such levels of the stem to produce numerous adventitious buds, irregularly arranged, while in the more distal levels of the stem, bud formation proceeds in a very definite and orderly manner.

4. *The Effect of Depressing Agents.*—It has been pointed out by us on numerous previous occasions that a certain relation exists between metabolic rate and depressing agents, such that regions of higher metabolic rate are more affected by depressing agents than regions of lower metabolic rate. If this general statement is correct, and various lines of evidence establish its accuracy, then it should be possible to reduce, eliminate, or reverse the differences in rate of regeneration that normally exist between apical and basal pieces. This is the case. Only two depressing agents were employed, ethyl ether and potassium cyanide. Apical and basal pieces of equal length were cut in the usual way and both exposed to the same concentration of these substances, made up in sea-water, for a certain length of time. The pieces were then thoroughly washed in several changes of sea-water and completed their regeneration in normal sea-water.

These experiments are presented in Tables IX. and X. In Table IX. are given the results of all the mass experiments performed with cyanide. They were performed in June, at room temperature, except number 22, which was placed in the refrigerator later. The concentration of cyanide used and the number of hours during which the pieces were exposed to it are

given in the table. In experiment 7, the concentration employed, 1/20000 mol., was too weak to produce any effect, but the effects of 1/10000 and 1/5000 mol. solutions are very striking. The rate of regeneration of both apical and basal pieces is retarded, but that of the apical pieces, in accordance with the hypothesis, is more retarded so that the basal pieces regenerate on the whole the more rapidly.

TABLE IX.

RECORD OF MASS EXPERIMENTS ON THE RATE OF REGENERATION OF APICAL AND BASAL HALVES WHEN BOTH ARE EXPOSED FOR A NUMBER OF HOURS AFTER CUTTING TO THE SAME CONCENTRATION OF POTASSIUM CYANIDE.

Columns give numbers of hydranths emerged at hours indicated. Exp. 10 control for exp. 13; exp. 21 for exp. 22. See Table III.

Exp. 7. KNC 1/20000 Mol. for 20 Hrs. After Cutting.			Exp. 13. KNC 1/10000 Mol. for 12 Hrs. After Cutting.			Exp. 22. KNC 1/5000 Mol. for 9 Hrs. After Cutting.		
Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*
36	1	0	60	1	0	84	1	0
40	2	0	62	2	2	93	2	0
46	5	2	*69	3	7	put into refrigerator		
48	11	3	72	8	9			
50	13	8	74	12	14	178	2	1
*58	18	16	76	13	16	205	7	3
61	22	18	78	15	20	210	8	7
64	23	20	80	17	20	216	8	9
67		21	83	20	23	*227	11	13
73		23	85	24	24	229	11	14
			*93	34	36	231	12	16
			101	35	40	237	13	20
			106	35	41	*249	16	22
			108	36	42	256	17	22
			111	37		264	19	22
			*120	38		*273	23	23
						276	24	23
						280	24	26
						283	24	29
						287	26	29
						*297	28	30
						300	29	
						305	30	

The results of the experiments with ether are given in Table X. Experiments 14 and 20 are mass experiments; 18 and 46 give records of the number of hours required for the regeneration of each piece. Experiments 14, 20, and 18 were performed in June at room temperatures, except that exp. 20 was kept in the refrigerator for part of the time after regeneration had begun. Experiment 46 was performed in December at a temperature of

12° C. ± 2. It will be observed that in the case of the mass experiments, the basal pieces on the whole regenerate more rapidly than the apical pieces, although both are retarded as compared with the controls. The individual experiments bring out the same point. The number of deaths was considerable but of 39 pairs in which both pieces regenerated, the basal pieces preceded the apical pieces in 14 cases, or 38 per cent. as compared with the result under normal conditions as given in Table V., where but 8 per cent. of the basal pieces precede the apical pieces.

A number of interesting points are brought out by these experiments with ether and cyanide. In the first place the rate of regeneration is greatly retarded. In the case of cyanide, where different concentrations were employed, the retardation is proportional to the concentration used. This retardation is evidenced by both kinds of pieces, the apical pieces being, however, more retarded than the basal pieces, with the consequence that the usual relation between the time of regeneration of apical and basal pieces is reversed. Now there can be little doubt that the rate of regeneration of pieces of *Tubularia* primarily depends upon the rate of chemical processes in those pieces. The effect of temperature upon the rate of regeneration is sufficient proof of this. Therefore, since depressing agents retard the rate of regeneration, it is impossible to doubt that they bring about this effect by lowering the rate of chemical reactions in the pieces. This is further evidenced by the fact that concentrations of these reagents which are effective at room temperatures are entirely without effect at temperatures of 12° and 13° C. Thus 1 per cent. ether is very effective at 20° C. but has no effect at 12° C. In order to alter the relations of apical and basal pieces at 12° C., it is necessary to use 2 per cent. ether. The same is true of cyanide. When, therefore, the metabolic rate is already lowered by low temperature, the action of depressing agents is diminished. This further supports the statement made at the beginning of this section that the action of depressing agents is related to the rate of chemical activity of the protoplasm which is exposed to them, and that such effects are greater the higher the metabolic rate of the living material. The differential effect, therefore, of ether and cyanide on the rate of regeneration of apical and basal

TABLE X.

RATE OF REGENERATION OF APICAL AND BASAL HALVES WHEN BOTH ARE EXPOSED FOR A NUMBER OF HOURS AFTER CUTTING TO THE SAME CONCENTRATION OF ETHER.

Experiments 14 and 20, mass experiments; experiments 18 and 46, individual experiments. Exp. 10, control for exp. 14; exp. 17 for 18 and 20; exp. 45 for 46; in Tables III. and V.

Mass Experiments.						Individual Experiments.					
Exp. 14, 1 % Ether for 12 Hrs. After Cutting.			Exp. 20, 1 % Ether for 12 Hrs. After Cutting.			Exp. 18, 1 % Ether for 15 Hrs. After Cutting.			Exp. 46, 2 % Ether for 17 Hrs. After Cutting.		
Hrs.	*a.*	*b.*	Hrs.	*a.*	*b.*	No.	*a.*	*b.*	No.	*a.*	*b.*
53	0	1	60	3	3	1	70	74	1	dead	dead
55	2	1	65	8	7	2	*87	115	2	dead	*137
59	5	4	67	8	12	3	*87	90	3	dead	dead
61	7	6	69	10	14	4	115	64†	4	dead	dead
*68	10	12	73	13	19	5	68	dead	5	91	160
71	10	14	75	14	19	6	74	94	6	100	91†
73	14	16	*83	16	25	7	92	dead	7	94.5	95
75	16	19	88	17		8	87	115	8	137	125†
77	20	23	90	19		9	99	dead	9	73	88
79	22	24	92	20		10	dead	99	10	89	79†
81	25	28				11	99	96†	11	113	127
83	26	29				12	74	70†	12	dead	125
*91	30	32				13	dead	dead	13	127	77†
94	31	34				14	dead	87	14	dead	dead
99	33	35				15	87	dead	15	71	91
101	36	36				16	dead	76	16	100	71†
106	36	36				17	94	89†	17	95	*137
*115	37	36				18	96	dead	18	74	*89
129	37	37				19	90	*107	19	*88	90
*140	37	38				20	63	63	20	68	dead
									21	*88	112
									22	90	100
									23	*88	*88†
									24	100	94†
									25	*88	90
									26	78	*116
									27	dead	115
									28	75	89
									29	89	77†
									30	dead	77
									31	69	dead
									32	77	91
									33	dead	77
									34	69	*111
									35	*111	87†
									36	91	101
									37	89	98
									38	*111	91†
									39	71	87
									40	dead	dead

Total number of regenerated pairs.....39
Number where *a* preceded.....24 or 61%
Number where *b* preceded.....14 or 38%
Number where *a* and *b* equal..... 1

pieces indicates very clearly that the apical pieces have a higher rate of chemical activity. They are more affected by the depressing agents and more retarded.

5. *The Effect of Cutting the Distal End of the Apical Piece at the Base of the Hydranth.*—When questioned regarding his manner of cutting the pieces for his experiments, Banus replied as follows (I quote verbatim from his letter): "The most distal cut was usually made as near as possible to the hydranth without including any part of it. Other times more basal parts were used. No difference in the results was found." To two subsequent letter requesting more specific statements concerning this matter and asking for a diagram showing the exact relation of the most distal cut to the base of the hydranth, Banus returned no replies. The first sentence quoted leaves little doubt that Banus made his most distal cut just below the base of the hydranth, therefore including in the apical pieces, the little neck or stalk region of the hydranths. The rest of Banus's statement is too vague to merit any attention. What is meant by "more basal parts"? How is one to know in the experiments reported by Banus in which cases the distal cut was made at the base of the hydranth, and in which cases more basally? Certain it is that in some of Banus's pairs of pieces the apical piece emerges first, and in others the basal piece. This indicates some great irregularity in his method of procedure. Probably those cases where the apical pieces emerged first are the ones in which "more basal parts were used." In the absence of more definite information, speculation is idle. We are here concerned with the fact that *usually the distal cut was made at the base of the hydranth.*

I have performed three experiments in which the apical pieces were cut in the manner usually employed by Banus and as represented in text-figure 5. Such apical pieces include the stalk of the hydranth. This stalk is incapable of regeneration. It together with that portion of the cœnosarc which occupies the distal end of the perisarc dies away and disintegrates. This process of death and disintegration of the apical end of apical pieces cut in this manner naturally delays the regeneration of the apical pieces, because regeneration does not begin until the end of the piece has rounded off and become covered with a layer of

cells. But this is not the only retarding factor in such pieces. The cœnosarc after the death of the apical end withdraws into the perisarc leaving a short apical region of empty perisarc. This empty perisarc crumples to a greater or less extent. Therefore when the hydranth does regenerate it has to push out through this empty region before it can unfold, and this of itself would further delay the time of emergence of the oral hydranth; but to make matters worse, the crumpling of the empty perisarc renders it very difficult for the hydranth to push its way to the surface. On account of all of these factors, the regeneration of the apical pieces is very greatly delayed when they are cut in the manner employed by Banus. In fact, in many cases, the oral hydranth is so greatly retarded that the aboral hydranth emerges first, and in a few cases, the oral hydranth never emerged on such pieces, a complete reversal of polarity with disappearance of the primordium of the oral hydranth having been observed. Presumably Banus failed to notice whether oral or aboral hydranths had emerged, but the two ends of such pieces are easily distinguished by the bit of empty perisarc so that there is no doubt of the correctness of my statements. Not only are the oral hydranths of these pieces delayed but they are often abnormal in appearance; they are enlarged and distended, owing probably to the pressure to which they are subjected in being forced out through the crumpled perisarc, and their tentacles are short and stumpy. They regulate to normal within a few hours after they have emerged. In two or three cases, partially doubled hydranths were produced.

The three experiments performed with pieces cut in the way employed by Banus and as represented in Fig. 5 are presented in Table XI. They were performed in December and regenerated at a temperature of 12° C. ± 2. Experiment 31 consisted of pieces 5–8 mm. long, the other experiments of pieces 10–12 mm. long. In connection with experiments 39 and 49, the number of both oral and aboral hydranths emerged on the apical pieces at each observation is given. These records include of course only those cases in which the aboral hydranths emerged first. In some of these cases the oral hydranths subsequently emerged, and this is indicated by the number in parenthesis which follows

the number of pieces having aboral hydranths. In experiment 49, six such pieces had failed to give rise to oral hydranths when the experiment was concluded and in three of these cases, no primordia of the oral hydranths were present, the polarity having been completely reversed.

The data given in Table XI. show in a very striking manner that the regeneration of apical pieces cut so that their distal ends

TABLE XI.

RECORD OF MASS EXPERIMENTS ON THE RATE OF REGENERATION OF APICAL AND BASAL PIECES OF EQUAL LENGTH WHEN THE DISTAL END OF THE APICAL PIECES IS TAKEN AT THE BASE OF THE ORIGINAL HYDRANTHS.

Exp. 31.			Exp. 39.				Exp. 49.			
Hrs.	*a.*	*b.*	Hrs.	*a* Oral.	*a* Aboral.	*b.*	Hrs.	*a* Oral.	*a* Aboral.	*b.*
60	1	2	62	5		0	61	0		1
*69	8	19	64	12		2	65	3		2
71	12	23	66	16		23	67	7		8
73	13	28	68	25		38	69	8		15
76	16	33	70	29		43	71	13		19
78	21	39	72	31		44	73	22	1	28
80	28	47	75	34		45	75	25	2(1)	36
82	33	49	*82	42		47	*85	31	4(1)	46
84	38	49	85	43	2	48	87	32	7(1)	47
87	44	49	87	45	3	49	89	34	8(1)	49
*94	47	52	89	48	3	50	91	35	10(2)	49
101	48		91	48	3	51	93	38	10(2)	49
103	49		95	49	3	51	96	40	10(2)	49
105	50		*107	50	3	52	98	40	10(2)	50
107	52		109	51	3(1)		*110	44	10(4)	
			111	52	3(3)					

are just at the base of the original hydranths is markedly delayed and that in the majority of cases, the basal pieces regenerate first. It should be remarked that the delay is chiefly in the time of emergence of the oral hydranths and not in its formation; for the primordia of the hydranths in these apical pieces form in advance as a rule of those of the corresponding basal pieces; but these hydranths can not emerge as rapidly owing to the fact that they must be pushed through the piece of empty crumpled perisarc left by the death of stalk region.

The data in Table XI. furnish the explanation of Banus's results. It is obvious that anyone who practices the method of cutting the apical pieces described in connection with this table and who mixes up such a method with procedures where "more

basal parts are used" can expect nothing but irregular and inexplicable results. By using such methods and failing to describe them it is possible to accumulate data which appear to contradict everything that previous workers have obtained. As long as no one takes the trouble to inquire by what procedures such data were obtained and as long as the author refuses to furnish any information about his methods, such data might stand on record indenfiitely in the scientific journals to puzzle future investigators. I believe that I have conclusively shown that Banus's data are completely invalidated by his experimental method. This work and that of previous investigators—Driesch, Morgan, Child, Stevens, and Allee—demonstrate incontestably that in *Tubularia* when other factors are equal the rate of regeneration of pieces of equal size depends upon the level which they occupied in the intact stem; it is more rapid the nearer the pieces lie to the original distal end of the stem. A metabolic gradient exists in the stem of *Tubularia* which is the primary cause of these regional differences in rate of regeneration.

H. Summary.

1. This experimental work was undertaken as a reply to a paper published by Banus ('18).

2. The existence of a metabolic gradient in the stem of *Tubularia* is demonstrated in this paper in four different ways.

(*a*) Differential susceptibility of apical and basal regions of the stem to ether and cyanide. Apical regions are more susceptible.

(*b*) Differential capacity of apical and basal regions to reduce potassium permanganate. The apical end of the organism has the greatest reducing power.

(*c*) Difference in electrical potential along the stem. Apical regions are electronegative (galvanometrically) to basal levels within the limits of the individual. (At a certain distance from the original hydranth of *Tubularia* a new individual is arising and the apical end of this is likewise electronegative to regions anterior to its level.) Since in general electronegativity is associated in protoplasm with increased oxidative metabolism, this difference in electrical potential along the stem of *Tubularia* is

evidence that distal levels have a higher metabolic rate than proximal levels.

(*d*) Difference in the rate of regeneration of apical and basal pieces. Work upon this point constitutes the bulk of the paper and is summarized under the subsequent heads.

3. Apical halves of the stem of *Tubularia* regenerate oral hydranths markedly faster than basal halves. In cutting such pieces it is essential to discard the original hydranth and the first millimeter or two of the stem; the remaining stem is then cut into two equal halves. The difference between such halves has been demonstrated by:

(*a*) Mass experiments in which all of the apical halves have been placed in one dish, the basal in another. In such cases, the number of apical pieces which have regenerated oral hydranths is nearly always in excess, rarely equal to, and never less than the number of basal pieces which have regenerated.

(*b*) Individual experiments, in which the number of hours required for the emergence of the oral hydranth on each piece was recorded. The apical pieces regenerated oral hydranths first in 91 per cent. of the cases (122 pairs of pieces observed).

4. Apical pieces regenerate on the whole more rapidly than basal pieces, even when the latter are twice as long as the apical pieces. Such apical pieces must not however be less than 5 mm. in length. In pieces over 10 mm. in length, length has very little effect upon the time of regeneration; in pieces less than 10 mm. in length, the longer pieces regenerate faster than shorter ones having their distal ends at the same anterior level but this effect of length is not sufficient to overcome the influence of level except in very short pieces (under 5 mm.).

5. The difference in rate of regeneration of apical and basal pieces which exists under normal condition can be somewhat reduced by using stems bearing branches and cutting the apical piece above the branch and the basal piece below the branch. Since the first branch marks the limit of the *Tubularia* individual, the apical pieces above such branches are really the basal regions of the principal *Tubularia* individual, and the basal pieces below the branch are near the apical end of the second individual. In consequence of these relations, the difference between the

time of regeneration of such apical and basal pieces is less than is the case when pieces are cut from corresponding levels of stems without branches.

6. The difference in rate of regeneration of apical and basal pieces of the stem of *Tubularia* can be reversed by putting both sets of pieces for a certain time after cutting into appropriate concentrations of depressing agents like cyanide and ether. Under such circumstances the basal pieces regenerate in advance of the apical ones on the whole. This is due to the fact that depressing agents affect most strongly those regions having a higher rate of chemical activity. Since apical pieces have a higher metabolic rate than basal pieces, they are more affected by the same concentration of depressing agent and hence their regeneration is more retarded. In such cases the basal pieces regenerate the more rapidly. That this explanation is correct is further evidenced by the fact that the action of depressing agents is greatly influenced by temperature. At lowered temperatures a higher concentration of the agent must be employed to obtain the same effect produced at higher temperatures by lower concentrations.

7. These results are in accord with those obtained by a number of previous investigators and are directly opposed to the results presented by Banus. Banus claims that there is no difference on the average between the time of regeneration of oral hydranths on apical and basal pieces of the stem of *Tubularia*. Personal communication with Banus has elicited the fact that his usual method of cutting the apical pieces was erroneous. He cut them in such a way that the distal end of the apical piece was taken just below the base of the hydranth. In such cases, as shown in this paper, the distal ends of the apical pieces die away and the regeneration and time of emergence of the oral hydranth on these pieces is greatly delayed. It is believed that Banus's results are invalidated by such a method of procedure.

8. The results presented in this paper together with those of others quoted in the paper show that the rate of regeneration of pieces of *Tubularia* depends, when other factors are equal, upon the level which those pieces occupied in the intact stem; it is more rapid the nearer the pieces lie to the original distal end of

the stem. A metabolic gradient exists in the stem of *Tubularie* which is the primary cause of these regional differences in rata of regeneration.

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